

Elevated Levels of Monocyte Activation Markers Are Associated With Subclinical Atherosclerosis in Men With and Those Without HIV Infection

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Background. Heightened immune activation among human immunodeficiency virus (HIV)-infected persons may contribute to atherosclerosis. We assessed associations of serologic markers of monocyte activation, soluble CD163 (sCD163) and soluble CD14 (sCD14), and monocyte chemoattractant protein 1 (CCL2) with subclinical atherosclerosis among men with and those without HIV infection in the Multicenter AIDS Cohort Study.

Methods. We performed noncontrast computed tomography on 906 men (566 HIV-infected men and 340 HIV-uninfected men), 709 of whom also underwent coronary computed tomographic angiography. Associations between each biomarker and the prevalence of coronary plaque, the prevalence of stenosis of $\geq 50\%$, and the extent of plaque were assessed by logistic and linear regression, adjusting for age, race, HIV serostatus, and cardiovascular risk factors.

Results. Levels of all biomarkers were higher among HIV-infected men, of whom 81% had undetectable HIV RNA, and were associated with lower CD4⁺ T-cell counts. In the entire population and among HIV-infected men, higher biomarker levels were associated with a greater prevalence of coronary artery stenosis of $\geq 50\%$. Higher sCD163 levels were also associated with greater prevalences of coronary artery calcium, mixed plaque, and calcified plaque; higher CCL2 levels were associated with a greater extent of noncalcified plaque.

Conclusions. sCD163, sCD14, and CCL2 levels were elevated in treated HIV-infected men and associated with atherosclerosis. Monocyte activation may increase the risk for cardiovascular disease in individuals with HIV infection.

Keywords. human immunodeficiency virus; inflammation; monocyte activation; atherosclerosis; plaque.

AIDS-related morbidity and mortality have declined with advances in the treatment of human immunodeficiency virus (HIV) infection [1], and age-related diseases, such as cardiovascular disease (CVD), have emerged as leading causes of death among HIV-infected individuals [2–4]. In addition to higher rates of acute coronary events [5–7], HIV-infected individuals have more subclinical coronary atherosclerosis and endothelial dysfunction than HIV-uninfected persons [8–10].

Systemic inflammation, including monocyte activation and migration, contributes to atherogenesis [11]. Despite different biological mechanisms of action [12–14], circulating levels of soluble CD163 (sCD163) and soluble CD14 (sCD14), monocyte activation markers, and of monocyte chemoattractant protein 1 (CCL2), a proinflammatory chemokine, are reliable markers of inflammation [15–18]. Levels of all 3 biomarkers are also elevated in HIV-infected persons [13, 19, 20]. Higher levels of sCD163 are associated with coronary atherosclerosis in HIV-uninfected individuals [21] and, in some prior studies, in HIV-infected patients [22, 23]. While some studies of HIV-infected patients have demonstrated associations between sCD14 and CCL2 and subclinical coronary and carotid atherosclerosis [13, 20], others have not [22, 24]. The role of these biomarkers in coronary atherosclerosis among HIV-infected individuals requires further clarification.

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The purpose of this study was to evaluate associations between serum levels of sCD163, sCD14, and CCL2 and subclinical coronary atherosclerosis among a large group of well-characterized men with and those without HIV infection and to determine whether HIV infection modifies these relationships. Subclinical coronary atherosclerosis, which is associated with an increased risk of cardiac events [25, 26], was assessed using noncontrast cardiac computed tomography (CT), to measure coronary artery calcium levels, and coronary CT angiography, to measure coronary artery stenosis and the extent and composition of coronary plaque [27–30].

METHODS

Population

The Multicenter AIDS Cohort Study (MACS) is an ongoing observational cohort study of HIV infection among men who have sex with men (MSM) conducted at 4 sites in the United States [31]. As reported elsewhere [9], a subset of MACS participants were eligible to undergo cardiac noncontrast CT if they were aged 40–70 years, had no prior history of cardiac surgery or percutaneous transluminal coronary angioplasty or coronary intervention, and weighed <136 kg. Coronary CT angiography was performed on men without contrast allergy, atrial fibrillation at the time of coronary CT angiography, or impaired renal function (estimated glomerular filtration rate, <60 mL/minutes/1.73 m²) within 30 days of scanning or during any previous MACS examination. All participants provided written informed consent, and human experimentation guidelines of the US Department of Health and Human Services and each institution were followed in the conduct of this clinical research. The study protocol was approved by the institutional review board at each institution.

Demographic Information and Laboratory Tests

MACS participants return every 6 months for standardized MACS research visits. Data on CVD risk factors and HIV clinical variables obtained by questionnaire, physical examination, and blood tests performed at the study visit closest to the CT visit (generally within 6 months) were used for this analysis. Race was self-reported at enrollment. Serum levels of glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured in fasting blood samples. Levels of low-density lipoprotein (LDL) cholesterol were calculated using the Friedewald equation or were measured directly in men with either a triglycerides level of >400 mg/dL or a nonfasting blood sample. Serum levels of sCD163 and plasma levels of sCD14 and CCL2 were measured in samples collected on the day of CT and stored at –70°C. Testing was done at the University of Vermont Laboratory for Clinical Biochemistry Research (Burlington), using a sCD163 enzyme-linked immunosorbent assay (ELISA; R&D Systems; interassay coefficient of variation

[CV] range, 1.71%–5.80%), sCD14 ELISA (R&D Systems; interassay CV range, 3.96%–5.22%), and a Luminex-based single-plex cytokine panel (catalog no. MPXHCYTO60K-01; Millipore; interassay CV range, 3.23%–4.06%) for CCL2.

Measures of HIV disease activity, including CD4⁺ T-cell counts and plasma HIV RNA levels (determined by the Roche ultrasensitive assay, with a limit of detection of 50 copies/mL), were obtained at each MACS visit. The duration of highly active antiretroviral therapy (HAART) was calculated on the basis of self-reported medication use at each MACS visit, and the history of clinically defined AIDS illnesses was determined by medical record confirmation of self-reported outcomes.

CT Acquisition and Analysis Methods

These procedures have been described previously [32]. Briefly, β -blocker (or calcium channel blocker) therapy and sublingual nitroglycerin were administered, unless contraindicated, prior to 64-detector or 320-detector CT. Noncontrast cardiac CT was performed, and coronary artery calcium scores were computed using the Agatston method [33]. Low-dose, prospective electrocardiogram-triggered protocols were used when applicable, with a median effective radiation dose of 1.9 mSv (interquartile range, 1.7–2.7 mSv) for the coronary CT angiography. CT images were transferred to the core CT reading center (Los Angeles Biomedical Research Institute at Harbor-UCLA) and analyzed by trained, experienced readers blinded to participant characteristics. The modified 15-segment model of the American Heart Association [34] was used to assess coronary CT angiography images for the presence, size, and composition of coronary plaque and the degree of stenosis in all assessable coronary segments. Plaque size for each segment was scored as follows: 0, no plaque; 1, mild; 2, moderate; or 3, severe. Segment stenosis was defined as follows: 0, no stenosis; 1, 1%–29% (minimal) stenosis; 2, 30%–49% (mild) stenosis; 3, 50%–69% (moderate) stenosis; or 4, \geq 70% (severe) stenosis. The total plaque score was calculated by summing the plaque size score for all assessable coronary segments. This method has been shown to be highly reproducible [35].

In addition, each coronary segment was classified as normal or as containing noncalcified, mixed, or calcified plaque. Noncalcified plaque was defined as any discernible structure that could be clearly assignable to the vessel wall, had a CT density less than the contrast-enhanced coronary lumen but greater than the surrounding connective tissue, and was identified in at least 2 independent planes. Mixed plaque was defined as any structure in which <50% of the plaque area was occupied by calcium. Calcified plaque was defined as any structure with a density >130 Hounsfield units that was visualized as separate from the intravascular lumen and discernable in at least 2 independent planes. The noncalcified, mixed, and calcified plaque scores for each participant were calculated by separately summing the scores for noncalcified, mixed, and calcified plaque across all segments for that participant [32].

Statistical Analysis

The distributions of demographic and clinical factors in men with and those without HIV infection were compared using the Wilcoxon rank sum test or the χ^2 test. Spearman rank correlation was used to assess correlations between the biomarkers. Because the biomarkers were not normally distributed and to avoid making assumption of linear effects, we categorized each biomarker into groups defined by quintiles. Values beyond the upper limit of detection were set to the upper limit of detection and included in the upper quintile, and those below the lower limit of detection were set to the lower limit of detection and included in the lowest quintile. Associations of each biomarker with age, race, and HIV serostatus were assessed using generalized logistic models with the quintiles as the outcome. Similar models were used for examining biomarker associations with CVD risk factors (body mass index [BMI]; cumulative pack-years of smoking; use of hypertension, diabetes, or lipid-lowering medications; systolic blood pressure; and fasting glucose and total and HDL cholesterol levels for those not receiving hypertension, diabetes, or lipid-lowering medications), adjusting for age, race, HIV serostatus, MACS study center, and time of MACS enrollment (before 2001 vs 2001 and later). Among HIV-infected men, associations of biomarker levels with current and nadir CD4⁺ T-cell count, undetectable plasma HIV RNA, percentage of visits with detectable HIV RNA (median, 17 visits [interquartile range, 14–23 visits]), years after HAART initiation, and history of AIDS were assessed, adjusting for demographic characteristics and CVD risk factors.

To examine the associations between the biomarkers and subclinical atherosclerosis, separate logistic regression models were run with the presence of plaque (plaque score, >0), coronary artery stenosis of $\geq 50\%$, and coronary artery calcium score of >0 as the outcomes and the biomarker categorization (quintiles) as predictor variables. Initial models adjusted for age, race, and HIV serostatus (limited adjustment) and subsequent models added CVD risk factors (full adjustment). Linear regression was used to assess the associations between quintiles of biomarkers and plaque extent (ie, plaque score) among individuals who had any plaque, for coronary artery calcium score and for total, calcified, mixed, and noncalcified plaque scores after limited and full adjustment. Plaque scores were natural-log transformed. Our primary results focus on the fully adjusted models, comparing quintile 5 (highest levels) with quintile 1 (lowest levels) of biomarkers for each outcome. A dose-response trend of each biomarker in association with plaque was assessed in secondary analyses by modeling the biomarker quintiles as a single continuous covariate in fully adjusted logistic or linear regression models. Multiple imputation was used to complete missing data on CVD risk factors (data on BMI were missing for 16 men; on pack-years of smoking, for 3; on hypertension medication use, for 4; on systolic blood pressure, for 30; on diabetes medication use, for 4; on fasting glucose level, for 22; on lipid-

lowering medication use, for 13; and on total and HDL cholesterol levels, for 17). For each outcome, multiple imputation included all predictors and the outcome. Missing values were imputed 5 times on the basis of the distribution of covariates, using the Markov chain Monte Carlo method [36] and assuming multivariate normality. Analyses were performed after stratification by HIV serostatus, and formal tests of significant interactions of the biomarkers, stratified by HIV serostatus, were done in unified models. Additional analyses among HIV-infected men were performed, adjusting for CD4⁺ T-cell count, detectable HIV RNA level, and history of AIDS. Further analyses excluded HIV-infected men with detectable HIV RNA. All statistical analyses were performed using SAS software (SAS Institute, Cary, North Carolina). Statistical significance was defined as a *P* value of < .05.

RESULTS

This analysis included 566 HIV-infected men and 340 HIV-uninfected men who had measurements available for all 3 biomarkers and who had undergone noncontrast cardiac CT imaging. Coronary CT angiography was performed in 426 HIV-infected men and 283 HIV-uninfected men (78.3% of all participants). As shown in Table 1, compared with HIV-uninfected men, HIV-infected men were younger; more likely to be African American; had lower BMIs; were more likely to be current smokers; had smoked for more pack-years; had lower systolic blood pressures; had lower total, LDL, and HDL cholesterol levels; and had higher fasting triglycerides levels. The majority (81%) of HIV-infected men had undetectable plasma HIV RNA (<50 copies/mL). The median duration of HAART use was 12.3 years, and 14.1% of HIV-infected men had a history of a clinical AIDS diagnosis.

Levels above the upper limit of detection for sCD163 were seen in 51 men (44 HIV-infected men and 7 HIV-uninfected men), and none were below the lower limit of detection. For sCD14, there was 1 HIV-infected man with levels above the upper limit of detection and 12 men (8 HIV-infected men and 4 HIV-uninfected men) with levels below the lower limit of detection. All men had CCL2 levels within the limits of detection. The sCD163 level was modestly correlated with sCD14 and CCL2 levels ($r = 0.23$ and $r = 0.17$, respectively), and the sCD14 level was modestly correlated with the CCL2 level ($r = 0.20$; all $P < .0001$). Levels of all 3 biomarkers were higher in HIV-infected men, compared with those in HIV-uninfected men (Table 1), and this difference persisted after exclusion of HIV-infected men with detectable HIV RNA and after adjustment for CVD risk factors (data not shown).

Among men with and those without HIV infection, higher levels of all 3 biomarkers were associated with older age and with selected cardiovascular risk factors; among HIV-infected men, higher levels of all 3 biomarkers were associated with lower current

Table 1. Characteristics of the Study Population, by Human Immunodeficiency Virus (HIV) Serostatus by Use of Contrast for Computed Tomographic (CT) Angiography

Characteristic	HIV Status			CT Technique(s)		
	Uninfected (n = 340)	Infected (n = 566)	<i>P</i> Value	Noncontrast and Contrast (n = 709)	Noncontrast Only (n = 197)	<i>P</i> Value
Age, y	55.7 ± 7.3	53.0 ± 6.5	<.001	53.7 ± 7.0	55.3 ± 6.6	.003
Race			<.001			.27
White	66.8	51.9		56.3	61.9	
African American	24.7	34.3		31.2	28.9	
Hispanic/other	8.5	13.8		12.6	9.1	
Hypertension	44.7	47.2	.38	45.7	55.5	.02
Systolic blood pressure, mm Hg	128.2 ± 15	125.8 ± 14.6	.02	126.5 ± 14.6	127.7 ± 15.5	.41
Hypertension medication use	32.4	36.2	.25	32.2	44.7	.001
Diabetes	9.7	12.9	.13	9.8	20.1	<.001
Diabetes medication use	7.4	9.4	.30	7.1	14.2	.002
Tobacco use			.001			.54
Never	23.8	24.4		24.4	24.7	
Current	20.6	31.3		26.8	30.4	
Former	54.7	43.1		48.9	44.8	
Smoking duration, pack-years	2.3 (0.0–21.1)	5.9 (0.0–22.3)	.03	4.7 (0–21.4)	4.3 (0–24)	.77
Body mass index ^a	27.3 ± 4.8	26.2 ± 4.5	.001	26.6 ± 4.5	26.8 ± 5.2	.95
Glucose level, mg/dL	96 (88–105)	98 (90–107)	.01	97 (89–106)	99 (92–110)	.002
Cholesterol level, mg/dL						
Total	191 (166–215)	186 (159–211)	.03	190 (163–213)	187 (160–213)	.67
LDL	112 (91–137)	102 (81–127)	<.001	107 (85–133)	103 (78–122)	.04
HDL	51 (43–61)	46 (38–55)	<.001	48 (40–58)	46 (39–58)	.28
Triglycerides level, mg/dL	103 (76–155)	134 (94–205)	<.001	119 (84–182)	135 (96–211)	.004
Lipid-lowering medication use	29.1	34.8	.07	32.3	36.1	.33
Serum creatinine level, mg/dL	1.0 (0.9–1.1)	1.0 (0.9–1.2)	.01	1.0 (0.9–1.1)	1.2 (1.0–1.4)	<.001
sCD163 level, ng/mL	547 (430–693)	680 (519–879)	<.001	603 (471–793)	708 (524–906)	<.001
sCD14 level, ng/mL	1282 (1114–1458)	1619 (1406–1899)	<.001	1455 (1228–1708)	1675 (1344–2009)	<.001
CCL2 level, pg/mL	236 (184–308)	276 (209–354)	<.001	256 (197–327)	288 (209–366)	<.001
HIV clinical factor		566		426	140	
HIV RNA level						
Undetectable ^b	...	81.0		80.0	84.1	.30
Detectable, copies/mL ^c		537 (131–12 200)		654 (123–16 200)	278 (182–2260)	.51
CD4 ⁺ T-cell count, cells/mm ³						
Overall	...	599 (426–751)		599 (426–756)	592 (431–748)	.95
Nadir	...	251 (144–335)		255 (155–339)	234 (117–319)	.10
HAART use						
Any		95.9		95.3	97.9	.19
Duration, y	...	12.3 (8.8–14.1)		12.1 (8.5–14.0)	13.0 (9.6–14.4)	.07
History of AIDS	...	14.1		11.5	22.1	.002

Data are mean ± SD, for normally distributed variables, or % of subjects or median (interquartile range) for nonnormally distributed variables. *P* values were obtained by the Wilcoxon rank sum test or the χ^2 test.

Abbreviations: HAART, highly active antiretroviral therapy; HDL, high-density lipoprotein; LDL, low-density lipoprotein; sCD14, soluble CD14; sCD163, soluble CD163; SD, standard deviation.

^a Calculated as the weight in kg divided by the height in m².

^b HIV RNA level of <50 copies/mL.

^c Among men with current detectable HIV RNA levels (>50 copies/mL).

and nadir CD4⁺ T-cell counts and with having a detectable HIV RNA level (Table 2). sCD163 and sCD14 levels were also associated with overall viremic burden while receiving HAART, defined

as the percentage of visits with detectable HIV RNA. CT results are shown in Table 3. As previously reported, HIV-infected men had a higher prevalence of noncalcified plaque [9, 22].

Table 2. Odds Ratio (ORs) and 95% Confidence Intervals (CIs), Based on Multivariate Models of the Predictors of Monocyte Activation Markers

	sCD163		sCD14		CCL2	
	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value
Model A^a						
Age ^b	1.11 (1.06–1.16)	<.001	1.06 (1.02–1.11)	.005	1.07 (1.03–1.11)	.001
Race (relative to white)						
Black	1.39 (.77–2.48)	.27	0.79 (.42–1.48)	.46	2.00 (1.11–3.60)	.02
Hispanic and other	2.68 (1.10–6.54)	.03	0.53 (.23–1.24)	.14	2.08 (.98–4.45)	.06
HIV serostatus	5.34 (3.26–8.76)	<.001	41.30 (20.68–82.45)	<.001	3.52 (2.20–5.63)	<.001
Model B^c						
Body mass index ^d	1.06 (1.01–1.11)	.02	0.87 (.82–.92)	<.001	1.04 (1.00–1.09)	.08
Pack-years of smoking ^b	1.00 (.99–1.02)	.44	1.00 (.99–1.01)	.97	1.01 (1.00–1.02)	.16
Hypertension medication use	1.62 (1.02–2.59)	.04	1.03 (.63–1.68)	.90	1.16 (.73–1.83)	.53
Systolic blood pressure ^e	1.06 (.86–1.30)	.59	1.05 (.85–1.30)	.63	1.03 (.85–1.25)	.75
Diabetes medication use	2.06 (.92–4.61)	.08	2.66 (1.16–6.11)	.02	1.74 (.79–3.83)	.17
Glucose level ^f	1.08 (.92–1.27)	.33	1.07 (.91–1.27)	.42	1.15 (.98–1.36)	.09
Lipid-lowering medication use	0.63 (.38–1.03)	.06	1.13 (.67–1.91)	.64	1.34 (.82–2.17)	.24
HDL cholesterol level ^f	0.72 (.59–.86)	.001	1.09 (.93–1.29)	.29	0.79 (.67–.94)	.01
Total cholesterol level ^f	0.87 (.81–.94)	<.001	1.04 (.97–1.12)	.30	0.99 (.93–1.06)	.75
Model C^g						
CD4 ⁺ T-cell count ^h	0.80 (.71–.89)	<.001	0.82 (.73–.94)	.003	0.85 (.76–.97)	.01
CD4 ⁺ T-cell count nadir ^h	0.78 (.65–.93)	.007	0.68 (.54–.84)	<.001	0.75 (.62–.90)	.003
Undetectable HIV RNA level ⁱ	0.16 (.07–.39)	<.001	0.64 (.27–1.49)	.30	1.17 (.57–2.39)	.67
Percentage of visits with detectable HIV RNA ⁱ	1.26 (1.14–1.41)	<.001	1.14 (1.02–1.28)	.03	1.07 (.97–1.18)	.16
Years after HAART initiation ^b	0.99 (.92–1.06)	.75	1.03 (.96–1.11)	.41	1.00 (.93–1.07)	.89
History of AIDS	2.07 (.83–5.20)	.12	2.84 (.91–8.86)	.07	2.40 (1.00–5.73)	.05

Data present associations between each risk factor and the odds of the monocyte activation marker being in quintile 5 relative to quintile 1.

Abbreviations: CI, confidence interval; CT, computed tomography; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MACS, Multicenter AIDS Cohort Study; OR, odds ratio.

^a Model A adjusted for age, race, HIV serostatus, MACS study center, and time of MACS enrollment (before 2001 vs 2001 and later) among the entire population (n = 906).

^b Per 1-year increase.

^c Model B adjusted for variables in model A for each cardiovascular risk factor separately in the entire population (n = 906).

^d Per 1-unit increase.

^e Per 10-mm Hg increase.

^f Per 10-mg/dL increase.

^g Model C adjusted for age, race, MACS study center, and time of MACS enrollment (before 2001 vs 2001 and later), and cardiovascular disease risk factors among HIV-infected men (n = 566).

^h Per 100-cells/ μ L increase.

ⁱ HIV RNA level of <50 copies/mL.

^j From the initiation of HAART to the CT visit for HIV-infected men who initiated HAART or from HIV infection to CT visit for those who did not initiate HAART.

Associations Between Biomarkers and Prevalence of Coronary Atherosclerosis

In the overall cohort, higher sCD163 levels (quintile 5, compared with quintile 1) were associated with a higher prevalence of coronary artery plaque and stenosis, even after adjustment for CVD risk factors (Figures 1 and 2 and [Supplementary Table 1A](#)). The summary below focuses on CVD-adjusted analyses. The odds for the presence of coronary artery calcium, any plaque, mixed plaque, calcified plaque, and coronary stenosis of $\geq 50\%$ were all >2.0 for sCD163 levels, but no significant

associations were seen with noncalcified plaque. A significant dose-response effect was also noted, with increasing quintiles of sCD163 associated with all outcomes except noncalcified plaque in secondary analyses (Figures 1 and 2). There were no significant interactions between sCD163 and plaque by HIV serostatus.

In stratified analyses, among HIV-infected men, higher sCD163 levels were associated with a greater odds of prevalent coronary artery calcium, calcified plaque, mixed plaque, and coronary stenosis of $\geq 50\%$, after adjustment for CVD risk

Table 3. Assessment of Coronary Atherosclerosis by Computed Tomography, by Human Immunodeficiency Virus (HIV) Status

Variable	HIV Uninfected	HIV Infected	P Value
Noncontrast CT scan analyzed, no.	340	566	
Coronary artery calcium present ^a	51.2	52.1	.78
Coronary artery calcium score among those with calcium present (n = 469)	71 (23–237)	71 (20–189)	.58
Coronary CT angiography scan analyzed, no.	283	426	
Prevalence of coronary plaque	74.6	77.9	.30
Prevalence of calcified plaque	38.9	34.0	.19
Prevalence of noncalcified plaque	53.0	63.6	.005
Prevalence of mixed plaque	32.9	34.7	.61
Prevalence of any coronary stenosis of ≥50%	14.5	16.7	.44
Total plaque score	3 (2–6)	4 (2–7)	.45
Calcified plaque score	2 (1–4)	2 (1–3)	.26
Noncalcified plaque score	2 (1–3)	2 (1–4)	.13
Mixed plaque score	2 (1–4)	2 (1–3)	.11

Plaque variables are shown as median (interquartile range) or % of men with plaque, unless otherwise indicated.

Abbreviations: CT, computed tomography; HIV, human immunodeficiency virus.

^a Defined as an Agatston score >0.

factors (Figure 3). The estimated odds ratio for associations of elevated sCD163 levels with any plaque and with noncalcified plaque among HIV-infected men were also >1.0 but were not statistically significant. The sCD163 level was associated with the coronary artery calcium prevalence among HIV-uninfected men only after adjustment for age and race (Supplementary Table 1A).

Complete tables demonstrating the associations between each biomarker quintile with the prevalence of coronary plaque subtypes in limited and fully adjusted models and in analyses stratified by HIV serostatus are presented in Supplementary Tables 1–3. Results obtained when HIV-infected men with detectable HIV RNA were excluded from the analysis were similar to results obtained for the entire HIV-infected population (data not shown). Among HIV-infected men, results were also similar after adjustment for current CD4⁺ T-cell count, detectable HIV RNA level, and history of AIDS, in addition to age, race, and CVD risk factors (data not shown).

Higher levels of sCD14 (quintile 5, compared with quintile 1) were associated with a greater odds of coronary stenosis of ≥50% in the entire population (Figure 2) and among HIV-infected men (Figure 3). A significant dose-response relationship was also observed for these associations. In the overall

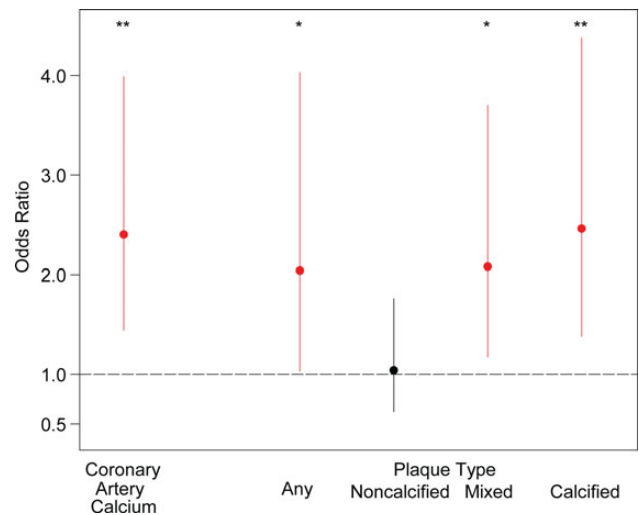


Figure 1. Estimated odds ratios (circles) and 95% confidence intervals (vertical lines) for the associations between the soluble CD163 level (sCD163; quintile 5, compared with quintile 1) and the prevalence of coronary plaque (defined as a plaque score of >0, as defined in “Methods” section) among the combined cohort (n = 906 for coronary artery calcium; n = 709 for coronary plaque subtypes). Models were adjusted for age, race, human immunodeficiency virus serostatus, and cardiovascular disease risk factors (body mass index; cumulative pack-years of smoking; use of hypertension, diabetes, or lipid-lowering medication; systolic blood pressure; fasting glucose level; and total cholesterol and high-density lipoprotein cholesterol levels for those not receiving hypertension, diabetes, or lipid-lowering medications). Red lines indicate P values of <.05. *P < .05 and **P < .01 for trends across biomarker quintiles.

cohort, higher sCD14 levels were not associated with noncalcified plaque, although results of the comparison of quintile 4 to quintile 1 were statistically significant (P = .04), and a dose-response was observed across the 5 quintiles (Supplementary Table 1B). Higher sCD14 levels were associated with the prevalence of coronary artery calcium and mixed plaque in HIV-uninfected men in fully adjusted models (Supplementary Table 1B). The interaction, stratified by HIV serostatus, for the association between sCD14 and the presence of coronary artery calcium was of borderline significance (P = .049), and there were no other significant interactions after stratification by HIV serostatus.

In the overall cohort, higher levels of CCL2 were associated with coronary stenosis of ≥50% (Figure 2). Among HIV-infected men, this association was significant with limited adjustment (P = .01), but attenuated and became borderline significant after adjusting for CVD risk factors (P = .05) (Figure 3). There were no other associations between CCL2 levels and atherosclerosis among HIV-infected men and no associations among HIV-uninfected men (Supplementary Table 1C). There were no significant interactions after stratification by HIV serostatus, and no dose-response relationships were observed.

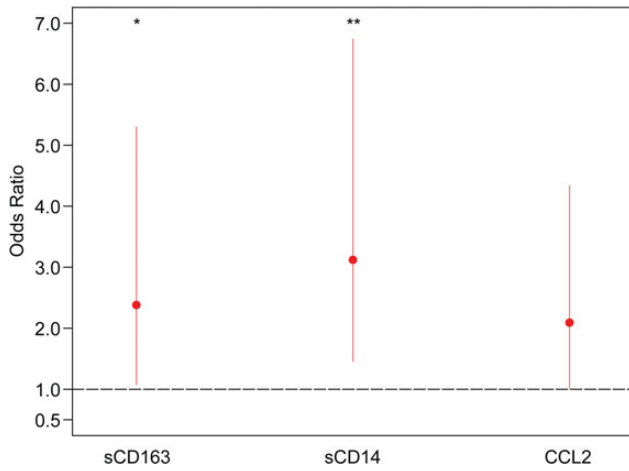


Figure 2. Estimated odds ratios (circles) and 95% confidence intervals (vertical lines) for the associations between biomarkers (quintile 5, compared with quintile 1) and prevalence of coronary artery stenosis of $\geq 50\%$ for the combined cohort ($n = 709$). See the Figure 1 legend for definitions of models and symbols. Abbreviations: sCD14, soluble CD14; sCD163, soluble CD163.

Associations Between Biomarkers and Extent of Coronary Atherosclerosis

In the overall cohort, a higher sCD163 level was associated with increasing coronary artery calcium score, with the highest sCD163 quintile associated with more coronary artery calcium than seen among persons with levels in the lowest quintile (Supplementary Table 2A). A significant dose-response relationship was seen between increasing quintiles of sCD163 and increasing coronary artery calcium score. A similar association with coronary artery calcium was also seen among HIV-infected men after adjustment for CVD risk factors. There were no significant interactions after stratification by HIV serostatus, but among HIV-uninfected men, sCD163 levels were not significantly associated with the extent of any plaque subtype.

Men with the highest sCD14 levels in the overall cohort had the greatest total plaque scores after adjustment for CVD risk factors (Supplementary Table 2B). No other associations between sCD14 level and plaque subtypes were significant.

Higher CCL2 levels were associated with greater extent of noncalcified plaque in the overall cohort and among HIV-infected men (Supplementary Table 2C). Significant dose-response relationships were also observed. There were no significant associations with any plaque subtypes among HIV-uninfected men, and no significant interactions after stratification by HIV serostatus.

As above, conclusions were not affected after exclusion of HIV-infected men with detectable HIV RNA or after adjustment for current CD4⁺ T-cell count, HIV RNA level, and history of AIDS, in addition to age, race, and CVD risk factors (data not shown).

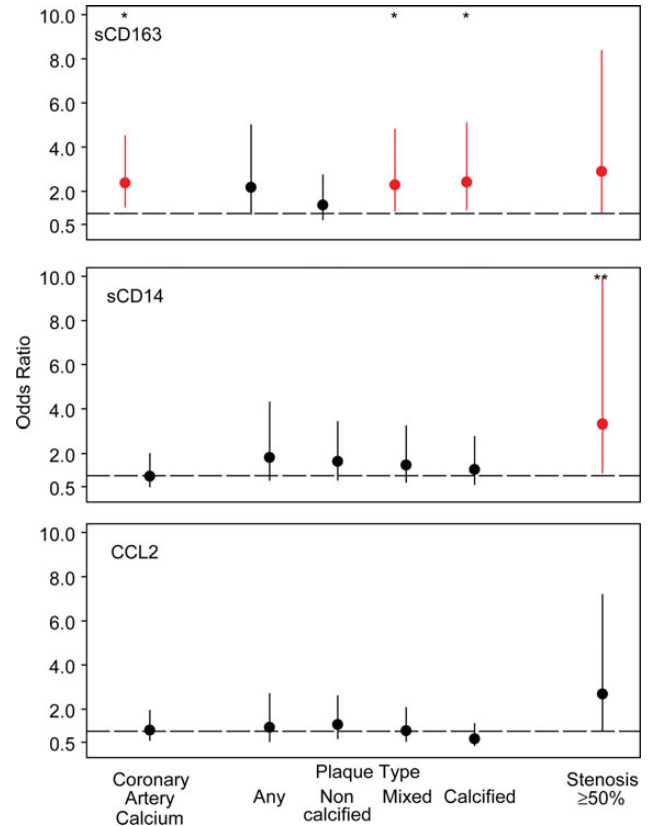


Figure 3. Estimated odds ratios (circles) and 95% confidence intervals (vertical lines) for the associations between biomarkers (quintile 5, compared with quintile 1) and prevalence of coronary plaque, including coronary stenosis of $\geq 50\%$, among human immunodeficiency virus-infected men ($n = 566$ for coronary artery calcium; $n = 426$ for coronary plaque subtypes). See the Figure 1 legend for definitions of models and symbols. Abbreviations: sCD14, soluble CD14; sCD163, soluble CD163.

DISCUSSION

This study is the largest conducted to date addressing the associations of monocyte activation markers with coronary atherosclerosis in demographically similar HIV-infected men and HIV-uninfected men. We found that levels of sCD163, sCD14, and CCL2 were elevated among HIV-infected men despite HIV suppression and were associated with lower CD4⁺ T-cell counts. These findings were not due to suboptimal treatment of HIV infection: 81% of the cohort had achieved virologic suppression, and the associations persisted after exclusion of viremic men and after adjustment for CD4⁺ T-cell count, HIV RNA level, and history of clinical AIDS. More importantly, we found that elevations in each of these 3 biomarkers were significantly associated with coronary artery stenosis, even after adjustment for traditional CVD risk factors. The most consistent associations were observed with elevated sCD163 levels, which were associated with a greater prevalence

of all plaque subtypes except noncalcified plaque. In contrast, elevated CCL2 levels but not elevated sCD163 or sCD14 levels were associated with a greater extent of noncalcified plaque. This finding is particularly relevant in light of our recent report demonstrating a greater extent of noncalcified plaque in HIV-infected men, compared with HIV-uninfected men, in our cohort [9]. CCL2 is a chemokine that is implicated primarily in initiating monocyte migration to the subendothelial space for atherosclerotic plaque formation [37]. Our future studies will assess longitudinal changes in coronary plaque subtypes to test whether elevated CCL2 levels in HIV-infected individuals may contribute to accumulation of noncalcified plaque and whether associations between elevated levels of sCD163 and sCD14 levels and plaque progression differ from associations with CCL2 levels.

Our findings in a large, well-phenotyped cohort are an important contribution to this area of research and support the results of other studies demonstrating associations between sCD163, sCD14, and CCL2 levels and atherosclerosis. Previous studies demonstrated that elevated levels of sCD163 and sCD14 were associated with subclinical carotid atherosclerosis [38], higher levels of sCD14 were associated with progression of carotid atherosclerosis [20] and with prevalent coronary artery calcium [39], and elevated levels of CCL2 and functional CCL2 genetic variants were associated with carotid and femoral artery atherosclerosis [13, 40] and thoracic aortic [41] atherosclerosis. Our findings differ from some other studies of HIV-infected participants that did not show associations between monocyte activation markers and atherosclerosis [24, 39]. Also, some studies [8, 22, 23] found associations between elevated levels of sCD163 and a greater burden of noncalcified coronary plaque, which was not found in our study. These discrepancies from our results are likely due to differences in participant characteristics, including age, and methods.

There are many strengths of our study, including the large, well-characterized cohort of HIV-infected men and also an HIV-uninfected comparison group of otherwise similar men at risk for HIV infection. We included multiple measures of monocyte activation and detailed assessment of coronary atherosclerosis, using coronary CT angiography to characterize both coronary artery stenosis and plaque composition. Limitations of the present study include the cross-sectional study design and inclusion of men only. Also, multiple comparisons were performed, and therefore some associations may represent false-positive results. We found few significant interactions after stratification by HIV serostatus, so results were presented for the overall cohort and after stratification by HIV serostatus, to demonstrate associations in the HIV-infected population. Since the sample size was smaller in the HIV-uninfected group, there were fewer statistically significant associations identified, but generally point estimates were similar between the 2 groups. In addition, we did not assess the impact of CCL2 genetic

variants on CCL2 levels or atherosclerosis in this study [42]; also of importance, although plasma and serum levels of CCL2 levels may vary, we measured plasma levels only [43].

In conclusion, elevated markers of monocyte activation were associated with subclinical coronary atherosclerosis independent of traditional cardiovascular risk factors in a large cohort of men with and those without HIV infection. Taken as a whole, the results of the present study support the hypothesis that monocyte activation, which is elevated in well-treated HIV-infected men, contributes to atherogenesis in this population. Subclinical atherosclerosis predicts the risk for CVD events in the general population [25, 44]. Although we found an association between elevated levels of sCD163 and coronary plaque and stenosis, a recent case-control study in HIV-infected persons found no association between sCD163 levels and subsequent myocardial infarction [45]. Our future analyses will assess associations with cardiovascular events in our cohort during the continuing follow-up period. Further studies are needed to define the potential role of measuring monocyte activation markers in clinical practice and to determine whether reducing monocyte activation, possibly via earlier treatment with antiretroviral therapy to prevent immunosuppression, can decrease the risk for CVD in HIV-infected individuals.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. R. T. G. has served as a consultant for ICON Medical Imaging. L. P. J. has served as a consultant on the 2013

CIV Cohorts Panel Advisory Board of Bristol-Myers Squibb. F. J. P. has served on the speakers bureaus of Gilead Sciences, Janssen Pharmaceuticals, Merck, and Bristol Myers Squibb. T. T. B. has served as a consultant for Gilead Sciences, Bristol Myers Squibb, Merck, Abbvie, EMD-Serono, and ViiV Healthcare. S. G. has served as a consultant for BMS/AstraZeneca, Navidea, Theratechnologies, and NovoNordisk. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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